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Assessment of the Effect of Adrenaline Toxicity, *Senna occidentalis L* and Propranolol in the Hearts of Wistar Rats

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Abstract
The biological response that occurs when animals are exposed to stress affects their well-being. In this study, the methanolic extract of S. occidentalis was assessed for its ameliorative action against Adrenaline (Adr) toxicity in experimental myocardial stress induced by the administration of Adr in rats. Rats were treated with adrenaline, 2mg/kg of body weight intraperitoneally at an interval of 24 hours for 5days. Adr produced microscopic changes such as Cardiac Hypertrophy, Congestion, Fatty change, Focal Dystrophic Calcification in the stressed group. Other changes observed includes; Interstitial Fibrosis, Fatty infiltration, Focal vascular calcific stenosis, Focal interstitial lymphocytic infiltrate and Focal vascular calcific stenosis. Treatment with S. occidentalis and Propranolol reduced the complications in the heart. The result was thus concluded that the complications from the heart injury may have resulted in toxicity affecting normal histology of cardiac muscle.

Keywords: Adrenaline, *Senna occidentalis*, Propranolol, Cardiac tissue, Histology

INTRODUCTION
The contribution of catecholamines in stress-induced heart injury is well documented (Navarro-Sobrino *et al.*, 2010). Catecholamines are not only essential for circulation physiology but are also important for several emergency situations, such as cardiopulmonary arrest and septic shock (Lu *et al.*, 2020). However, a dysregulated release of catecholamines may also lead to severe cardiac toxicities (Lu *et al*., 2020). Adrenaline being a frequently used catecholamine acts by stimulating alpha, beta-1, and beta-2- adrenergic receptor (Murthy *et al*., 2015). Though a lifesaving drug and an important pharmacological agent in acute management of cardiac

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arrest, but has adverse side effects, including angina, cardiac arrhythmia, and sudden death (Murthy *et al*., 2015; Nawaz-Khan *et al.*, 2018) . The dosages and routes of administration of adrenaline determine its toxicity (Murthy *et al*., 2015). Clearly, successful long-term survival from adrenaline administration requires balancing acute release while minimizing chronic exposure (Romero *et al*, 2007). It can thus be established that adrenaline exerts oxidative and nitrative stress in rats, increased damage to lipids and proteins, and damage of cardiomyocytes and cytogenetic damage (Radaković *et al*., 2018).

Adrenaline is a physiologically essential catecholamine for survival and an important pharmacological agent in acute management of cardiac arrest (Nawaz-Khan *et al*., 2018). However, recent studies have demonstrated that prolonged exposure to adrenaline exerts some detrimental effects on the body and cardiac physiology, resulting in adverse clinical outcomes (Nawaz-Khan *et al*., 2018). Several mechanisms account for the cardiotoxic effects (changes in cardiac contractile force, coronary pressure, dysrhythmias) are reviewed, to include: disturbances in cellular calcium homeostasis, redox cycling with the generation of free radicals, the depletion of oxygen with resultant hypoxia and the perturbation of oxidative phosphorylation (Behonick *et al*., 2001).

While adrenaline and noradrenaline (NE) are commonly used in emergency medicine, limited studies have discussed the harm of exogenously induced catecholamine overdose (Lu *et al*., 2020). We hypothesized that high concentration of adrenaline would result to severe stress. Stress increases the risk of cardiac diseases, including heart attack and sudden death (Sahin & Demirci, 2017). Up until now, changes in biochemical and physiological parameters occurring a long time after stress are not yet elucidated (Shkurashivska & Ersteniuk, 2019). It is known that excessive doses of catecholamines produce myocardial destruction with myocyte loss and necrosis, as well as extensive fibrosis (Izumi *et al*., 2009). Accordingly, the main aim of this study was to access the changes that occurred after the administration of adrenaline to experimental rats. Additionally, to assess the therapy of *S. occidentalis* and a standard β-blocker, propranolol on the stressed rats.

MATERIALS AND METHODS

Experimental Animals

Male wistar rats (35), weighing 150-180g were obtained from the Department of Physiology and Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria. The rats were housed in the Departments' Animal Center under standard laboratory condition in a room with 12-hour dark/light cycle. The rats were allowed free access to tap water and were fed with commercial grower's mash (PLS Feeds, Zaria, Kaduna State, Nigeria) and water *ad libitum* throughout the experiment. The animal care and experimental protocols were in accordance with nationally approved guidelines. The animals were deprived of food but allowed free access to tap water for 24 hours prior to experiments.

Source of Adrenaline

Adrenaline was obtained from Sigma Aldrich (St. Louis, MO, USA).

Preparation and Extraction

The seeds of *Senna occidentalis* was shade dried, roasted at 210^0 C for 15 mins (Olapade & Ajayi, 2016). The coarse powdered seeds were extracted with 2 L of 80% methanol in H_2O (v/v) by maceration. The extract obtained was evaporated to dryness.

Acute Toxicity Studies

Acute toxicity of *S. occidentalis* methanolic extract was carried out using modified Lorke's method (Lorke, 1983). The oral median lethal dose was calculated using the formula:
LD50=√minimum toxic dose × maximum tolerated dose.

Experimental Design

Experimental rats were starved of food and water for 24h and treated with 2 mg/kg body weight of adrenaline administered intraperitoneally (Radaković *et al*., 2018), once daily for five consecutive days. Repeated doses were administered to mimic stress condition in the animals. However, repeated dose administered was to ascertain whether consecutive increase in catecholamine levels resulted in Takotsubo cardiomyopathy. Since Takotsubo cardiomyopathy has been associated with high plasma catecholamine levels (Sachdeva *et al*., 2014). *Senna occidentalis* was used as a positive control, while the negative control group was treated with saline (0.9% NaCl). Propranolol was used as a positive control and 40 mg/kg body weight per rats was given. This was based on previous studies assessing the safety of the drug. Experimental rats were allocated into 7 groups (5 rats/per group).

- Groups 1: received normal saline only.
- Groups II: received Adrenaline (2 mg/kg body weight) treated rats (AIR).
- Groups III: Received adrenaline (2 mg/kg) and treated with propranolol (40mg/kg).
- Groups IV: received adrenaline (2mg/kg) and was treated with *Senna occidentalis* extract (125mg/kg).
- Groups V: received adrenaline (2mg/kg) and treated with *Senna occidentalis* extract (250mg/kg).
- Groups VI: received adrenaline (2 mg/kg) and was treated with *Senna occidentalis* extract (500 mg/kg).
- Groups VII: Normotensive rats that received *Senna occidentalis* extract (500 mg/kg). The extract was given for seven days.

Histopathological Examination

After the experimental period, the rats were sacrificed by cervical dislocation after an overnight fast. The samples were fixed and processed following standard histological procedures (Zhou *et al.,* 2008). Sections from the normal and treated hearts were excised from rats and immediately fixed in 10% buffered formalin. The ventricular mass was sectioned from the apex to the base of the heart, which was embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Five-micrometer thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin for the assessment of histopathological lesions. Pathological/cardiac changes were observed under light microscope and photomicrographs were taken at X200 (Zhou *et al.,* 2008).

Data Analysis

The extent of cardiac lesions was reviewed as Absent (N), Mild (+), Moderate (++) and Marked (+++).

RESULTS

Histological Changes in Cardiac Tissues Due to Adrenaline Induction and Treatment with Extract.

The heart of normal rats revealed normal cardiac muscle with no definite lesions. However, a disruption of myocardial architecture was present in the stressed hearts (Group II, III, IV, V, VI). Adrenaline-treated rats showed marked hypertrophy, marked fatty change, while

congestion was detected at the vasculature of the stressed animals (Group II), other changes include marked fatty infiltration. Treatment with propranolol (Group III) showed mild hypertrophy, fatty change and congestion. Moderate hypertrophy and fatty change, interstitial fibrosis was also observed in 125mg/kg extract given (Group IV), moderate hypertrophy and fatty change were also seen in 250mg/kg extract (Group V) together with fatty infiltration. The 500mg/kg body weight of extract group (Group VI) exhibited deleterious heart changes in hypertrophy together with focal dystrophic calcification at the interstitium, together with focal vascular calcific stenosis and focal interstitial lymphocytic stenosis. Cardiac tissues of Group VII showed fatty infiltrations. Treatment with propranolol almost corrected the adrenaline-induced heart changes as compared to rats of group IV and V. The current work thus revealed that Senna occidentalis extract at 125 and 250mg/kg has a healing effect on the cardiac complications associated with adrenaline

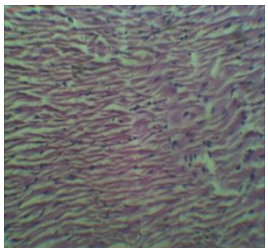
Table 1: Grading system used to score the histological damage.

+	**Mild**
++	**Moderate**
+++	**Marked**

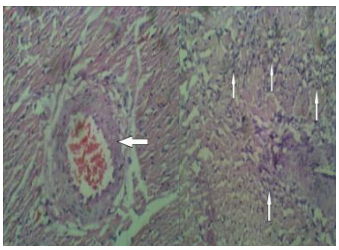
Table 2: Cardiac changes associated with adrenaline induction and the effect of propranolol and *Senna occidentalis*.

Animal Groups	Cardiac myocytes	Instertitium	Vasculature	Others
Group 1				
1	N	N	N	NIL
2				
4	N	N	N	NIL
5	N	N	N	NIL
Group II				
1	H+, FC+++	NIL	C+	FI+
2	H+++, FC0	NIL	NIL	NIL
3	H+++, FC++	NIL	C+++	FI+
4	H+++, FC0	NIL	NIL	FI+++
5	H+++, FC++	NIL	C++	FI+++
Group III				
1	H+, FC++	NIL	C+	NIL
2	H+, FC0	NIL	NIL	NIL
3	H+, FC0	NIL	NIL	NIL
4	H+, FC+	NIL	NIL	NIL
5	H+, FC+	NIL	C+	FI+
Group IV				
1	H++, FC0	IF+	C+	NIL
2	H++, FC0	NIL	NIL	NIL
3	H++, FC+	NIL	NIL	NIL
4	H++, FC0	NIL	C+	NIL
5	H++, FC+	NIL	C+	NIL
Group V				
2	H++, FC0	NIL	NIL	FI++
3	H++, FC0	NIL	NIL	FI++
4	H++, FC0	NIL	NIL	FI++
5	H++, FC0	NIL	C+	NIL
Group VI				
1	H++, FC0	NIL	NIL	Focal vascular calcific stenosis
2	H++, FC0	NIL	NIL	NIL
3	H+++, FC0	NIL	NIL	Focal interstitial lymphocytic infiltrate
4	H+++, FC0	Focal dystrophic calcification	NIL	NIL
5	H+++, FC0	NIL	C+++	Focal vascular calcific stenosis
Group VII				
1	H+, FC0		NIL	NIL
2	H0, FC0	NIL	NIL	NIL
3	H0, FC0	NIL	NIL	FI
4	H0, FC0	NIL	NIL	FI
5	H0, FC0	NIL	NIL	NIL
		NIL		

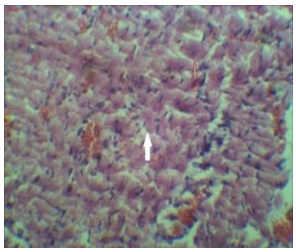
Note: N- Normal, H-Hypertrophy, C-Congestion, FC- Fatty Change, IF- Interstitial Fibrosis, FI- Fatty infilteration.



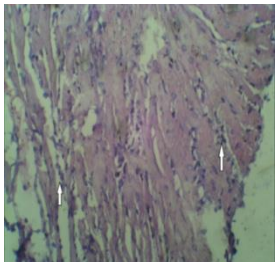
GRP I: Normal nucleus & Cytoplasm



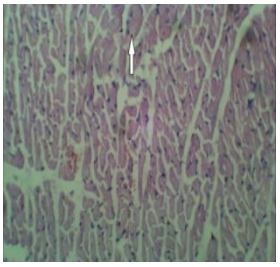
GRP II: Hypertrophy+++, Congestion++, Fatty Change+++, Fatty



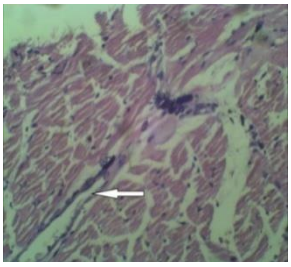
GRP III: Hypertrophy+, Congestion+, Fatty Infilteration+.



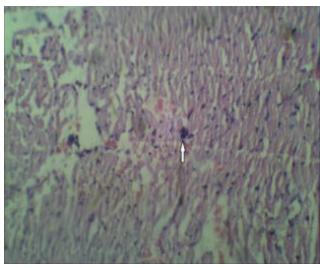
GRP IV: Hypertrophy++, Fatty change Interstitial fibrosis.



GRP V: Hypertrophy++, Fatty infiltration++.



GRP VI: Hypertrophy++, Focal Dystrophy calcification



GRP VII: Fatty infiltration

Plate: Transverse Sections of Cardiac Tissues from Group I-VII showing the Effect of Adrenaline and Treatment with Various Concentrations of *S. Occidentalis* Seeds Extract.

DISCUSSION

The advanced life support guidelines suggest the use of adrenaline during cardiopulmonary resuscitation. This increases perfusion pressure and coronary blood flow through its alpha-adrenergic peripheral vasoconstriction, allowing minimal rises in coronary perfusion pressure to make defibrillation possible (Carvalho *et al.*, 2012). In conflicting the alpha-adrenergic effects, adrenaline's β- stimulation may have detrimental effects through an increase in myocardial oxygen consumption and a reduction of subendocardial perfusion, leading to post resuscitation cardiac dysfunction (Carvalho *et al.*, 2012). Catecholamine-induced toxicity tends to reproduce many aspects of myocardial infarction, with various degrees of cardiomyocyte necrosis and apoptosis intimately associated with the infiltration of polymorphonuclear and mononuclear leukocytes (Wang *et al.*, 2017). Despite these differences, overlap does exist among categories, and histological features of myocardial injury from any etiology change over time (Wang *et al.*, 2017).

The reported clinical manifestations after adrenaline administration include hypertensive crisis followed by a fall in blood pressure, tachycardia, chest pain, myocardial ischemia, Cardiac arrhythmias, stenosis, headache, cyanosis and coldness of extremities, and pulmonary edema (Costa *et al.*, 2011). Besides some of these manifestations occurred in patients even after using the therapeutically recommended doses of adrenaline (Costa *et al.*,

2011). Consequently, the indication of stress as a result of adrenaline treatment increases the risk of cardiac diseases, including heart attack and sudden death (Sahin & Demirci, 2017).
In previous study, vascular changes, which are presented as congestion, haemorrhage and interstitial oedema; acute degenerative changes, attributed to cardiotoxicity by catecholamines and consisted of contraction band necrosis, wavy fibres, cytoplasmic hypereosinophilia and perinuclear vacuolisation; as well as the presence of interstitial myoglobin globules and infiltration of inflammatory cells were present as major cardiac lesions (Câmara et al., 2019). However, the major cardiac lesions associated with stress in this study are cardiac hypertrophy, congestion and fatty infiltrations. Thus, the infiltrate consisted predominantly of fatty infiltrations, others include focal vascular calcific stenosis, focal interstitial lymphocytic infiltrate, which was almost certainly very acute. Pathological classification can be complicated, and the clinical history, laboratory outcomes, and other postmortem findings need to be taken into consideration before reaching a conclusion as to etiology (Wang *et al*., 2017). However, in this study, *S. occidentalis* extract could not moderate the amount of necrosis as it remained within the normal ranges.

Additionally, there was an improvement in the rats after treatment with beta-blocker. The mechanism of the beta blocker action could be hypothesized that acute mortality associated with sudden increase in catecholamine levels occurs due to malignant arrhythmias and beta-blockers increased survival by ablation of these lethal arrhythmias (Sachdeva *et al*., 2014). Several investigators (Kyuma *et al*., 2002; Yoshioka *et al*., 2008) have reported that propranolol, a β-blocker, may be very effective in patients who showed a significant intraventricular pressure gradient because of the activation of the sympathetic nerve. The efficacy of β-blockers may be attributed to the improvement of β-adrenergic signaling and to protection from catecholamine myocyte toxicity in the heart because of a severe increase in plasma catecholamine and the activation of the sympathetic nerve (Kyuma *et al*., 2002; Yoshioka *et al*., 2008) .

CONCLUSION

The treatment of *Senna Occidentalis* was not able to reverse the disorder and the study was able to provide a warning about the excessive exposure to exogenous adrenaline. The result obtained in this study imply that high-dose adrenaline administration was associated with severe adverse effects in the treatment of local anesthetic overdose, suggesting that there may exist a safety threshold dose for epinephrine in this setting. It can also be concluded that the chronic stress caused myocardial injury containing degeneration and oedema leading to cardiac damage. However, the basis of the stress-induced cardiac diseases could not been exactly explained yet.

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